



EP166-EN.01

CD73 Inhibitor Screening Kit

Pack Size: 96 tests

Catalog Number: EP-166

Important: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures

[HTTP://WWW.ACROBIOSYSTEMS.COM](http://www.acrobiosystems.com)

Intended Use

This kit is a 96-well colorimetric assay designed for screening and profiling applications of inhibitors of binding between human CD73 and AMP.

It is intended for research use only (RUO).

Materials Provided

TABLE 1. Materials Provided (pls modify according to COA)

Catalog	Components	Size (96 tests)	Format	Storage
EP166-C01	High-bind Plate	1 plate	Solid	2-8°C
EP166-C02	Human CD73	1µg	Powder	2-8°C
EP166-C03	AMP	5000µm/L×500µL	Powder	2-8°C
EP166-C04	AMP-CP (CD73 Inhibitor)	10000µm/L×30µL	Powder	2-8°C
EP166-C05	1xDilution Buffer	50mL	Liquid	2-8°C
EP166-C06	Malachite Green Reagent A	2mL	Liquid	2-8°C
EP166-C07	Malachite Green Reagent B	2mL	Liquid	2-8°C

Reagents/Equipment Needed but not Supplied

Single or dual wavelength microplate reader with 620nm filter ;

Centrifuge ;

37 °C Incubator ;

Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision ;

10 µL, 200 µL and 1000 µL pipette tips ;

Test Tubes ;

Graduated cylinder ;

Deionized or distilled water for dilution ;

Storage and Validity Instructions

Unopened kit should be stored at 2°C -8°C upon receiving. Find the expiration date on the outside packaging and do not use reagents past their expiration date..

The kit should be stored as TABLE 1 after the reconstitution of lyophilized materials. The shelf life is 30 days from the date of opening.

Note:

- a. Do not use reagents past their expiration date.
- b. Find the expiration date on the outside packaging.

Reagent Preparation

1. Restore all reagents and samples to room temperature (20-25°C) before use.
2. Reconstitute the provided lyophilized materials to stock solutions with sterile deionized water as recommended in Tab.2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortex. The reconstituted stock solutions should be stored at -70°C. **Avoid freeze-thaw cycles.**

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.	Key Point
EP166-C02	Human CD73	1µg	5µg/mL	200µL, water	After reconstruction, each tube should be subpackaged at least 80uL and stored at -70°C
EP166-C03	AMP	5000µm/L×500µL	5000µm/L	500µL, water	After reconstruction, each tube should be subpackaged at least 100uL and stored at -70°C
EP166-C04	AMP-CP	10000µm/L×30µL	10000µm/L	30µL, water	After reconstruction, it can freeze and thaw up to 3 times

Recommended Protocol

1. Add Samples

- 1) If you intend to use the provided AMP-CP as a reference (Std.), you can continuously dilute the AMP-CP 2x from 500µm/L to 0.22140625µm/L.
- 2) Add 25µL of Serial dilutions of AMP-CP or Other inhibitors to each well and leave a couple of wells for No-inhibitor Control.
- 3) Dilute Human CD73 from 5µg/mL to 0.05µg/mL with Dilution Buffer to make Human CD73 working solution and add 25µL Human CD73 working solution.
- 4) At the same time, immediately dilute AMP from 5000µm/L to 200µm/L and add 50µL to the reaction strip, seal the plate with microplate sealing film and incubate at 37°C for 1 hours.

FIG.1 PREPARATION OF 1:1 SERIAL DILUTIONS OF THE AMP-CP(CD73 Inhibitor)

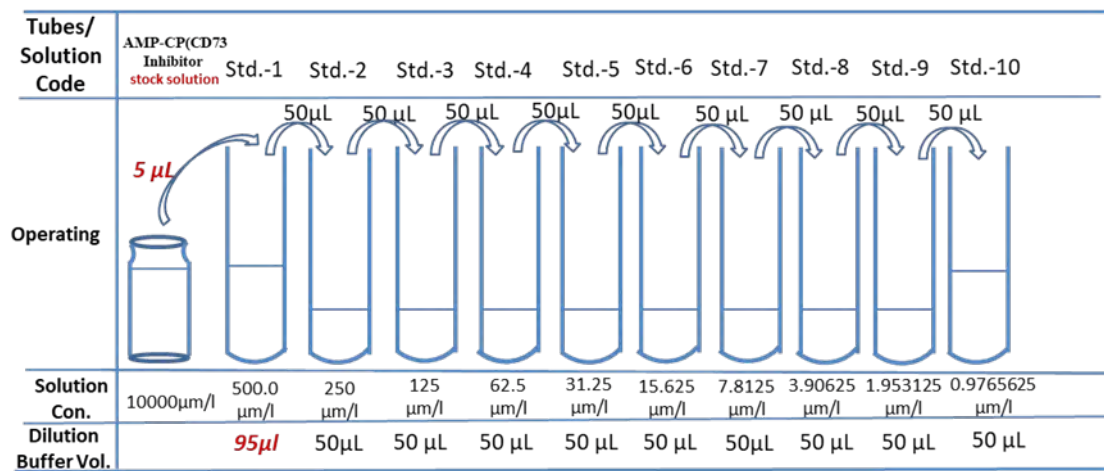


FIG.2 PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std.-1	Std.-9
B	Std.-2	Std.-10
C	Std.-3	Std.-11
D	Std.-4	Std.-12
E	Std.-5	Blank
F	Std.-6	Blank
G	Std.-7	Blank
H	Std.-8	Blank

2. Substrate Reaction

- 1) Add 20µL Malachite Green Reagent A and gently pat the enzyme plate and mix well, incubate at room temperature for 10 minutes.
- 2) Add 20µL Malachite Green Reagent B and gently pat the enzyme plate and mix well, incubate at room temperature for 20 minutes.

3. Data Recording

Read the absorbance at 620 nm using UV/Vis microplate spectrophotometer.

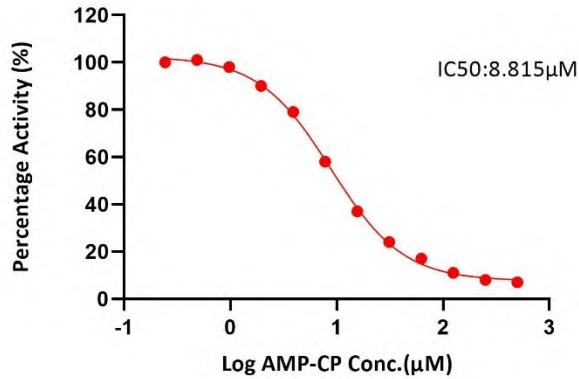
Precautions

- 1) This kit is for research use only and is not for use in diagnostic or therapeutic applications.
- 2) This kit should be used according to the provided instructions.
- 3) Do not mix reagents from different lots.
- 4) All reagents should be balanced to room temperature (20°C-25°C) before use.
- 5) This kit should be stored at 2°C-8°C.
- 6) Please prepare the working solution of each component according to the needs of the experiment.

Typical Data

INHIBITION OF CD73: AMP BINDING BY AMP-CP

Serial dilutions of AMP-CP (1:1 serial dilution, from 500µm/L to 0.244140625µm/L) was added into CD73: AMP binding reactions. The assay was performed according to the protocol described below. Background was subtracted from data points prior to log transformation and curve fitting.



AMP-CP Con.(µm/L)	OD620	Percentsg
500	0.136	7%
250	0.158	8%
125	0.217	11%
62.5	0.329	17%
31.25	0.474	24%
15.625	0.726	37%
7.8125	1.137	58%
3.90625	1.555	79%
1.953125	1.77	90%
0.9765625	1.941	98%
0.48828125	1.992	101%
0.244140625	1.982	100%
0	1.975	100%

For

each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The example data is for reference only.